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Development and validation of an extensive stochastic model for the simultaneous growth of *Listeria monocytogenes* and lactic acid bacteria – A case study with naturally contaminated cold smoked Greenland halibut

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OBJECTIVE

The objective of the present study was to develop and validate a combined stochastic model for growth of *Listeria monocytogenes* and lactic acid bacteria (LAB).

METHODS

An existing deterministic model for the simultaneous growth of *L. monocytogenes* and LAB (Mejlholm and Dalgaard, 2007, 2009) was expanded into a stochastic model using the Analytica software. The stochastic model includes the effect of 12 environmental parameters as well as their interactive effects, together with the effect of microbial interactions between *L. monocytogenes* and LAB. To evaluate the performance of the stochastic model, a total of 24 storage trials with naturally contaminated cold-smoked Greenland halibut (CSGH) was carried out at 5, 8 and 12 °C. Samples were supplied by a Danish seafood processor from a withheld batch of CSGH being positive for *L. monocytogenes*. The variability in product characteristics was determined by analysis of five samples. In addition, 56 samples of CSGH were collected from the same company during a period of 12 months to obtain a more thorough description of the variability in product characteristics and in the occurrence of *L. monocytogenes* and LAB. In contrast to traditionally produced CSGH, all the examined samples were added acetic and lactic acids in order to improve the safety of the product.

RESULTS

Product characteristics of CSGH used for the storage trials were: Water phase salt ($3.31 \pm 0.41\%$), pH (6.13 ± 0.15), phenol (10.1 ± 0.8 ppm), water phase acetic acid (3011 ± 472 ppm) and water phase lactic acid (7657 ± 1162 ppm). The initial concentration of *L. monocytogenes* in CSGH was 0.13 ± 0.28 log (cfu/g), with all samples being positive. No growth of *L. monocytogenes* was observed at 5°C whereas an increase in the concentration was seen for some of the storage trials at 8 and 12°C, with the maximum population density (MPD) of the pathogen reaching approx. 1.7 log (cfu/g) at both temperatures. By including a relative lag time (RLT) of 4.5 for *L. monocytogenes* (Ross, 1999), MPDs of 1.3, 1.6 and 3.2 log (cfu/g) were predicted by the stochastic model at 5, 8 and 12°C, respectively. Without a RLT of 4.5, the corresponding predictions were 2.8, 3.6 and 4.2 log (cfu/g). Product characteristics of the 56 samples of CSGH were: Water phase salt ($3.10 \pm 0.53\%$), pH (6.12 ± 0.16), phenol (10.4 ± 4.2 ppm), water phase acetic acid (3586 ± 1061 ppm) and water phase lactic acid (9701 ± 1954 ppm). Assuming a shelf life of 28 days at 5 °C, *L. monocytogenes* was predicted to grow to no more than 1.5 log (cfu/g) in CSGH. Without a RLT of 4.5, the corresponding prediction was 3.4 log (cfu/g). Predicting growth of *L. monocytogenes* in CSGH without addition of acetic and lactic acids resulted in a MPD of 3.3 log (cfu/g) and more than 25% of the samples were estimated to exceed the regulatory limit of 2.0 log (cfu/g).

CONCLUSIONS AND IMPACT OF THE STUDY

It was clearly demonstrated that to accurately predict the MPD of *L. monocytogenes* in naturally contaminated CSGH both the lag time and the effect of microbial interaction needs to be included. Without these effects the MPD of *L. monocytogenes* was predicted to be up to 100.000 times higher than observed and high percentages of samples were estimated to exceed 2.0 log (cfu/g). Furthermore, it was shown that CSGH with added acetic and lactic acids complied with the EU-regulation (EC 2073/2005) on *L. monocytogenes*, even when the variability in product characteristics was taken into account. Without the addition of acetic and lactic acids, CSGH constitutes a high-risk product with the potential of causing listeriosis.

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